

CLAIMS

1. A high throughput screening method for assaying non-,
pro- or anti-apoptotic or proliferative or necrotic
activity of test compounds in cells using vectors
coding for a marker protein and cells transfected with
said vector comprising a primary and a secondary
screening step.
2. The screening method of claim 1, being industrially
applicable.
3. The screening method of claim 2, wherein the marker
protein is the GFP protein or GFPmut1 protein.
4. The screening method of claim 3, wherein the test cells
are derived from healthy individuals/animals or
patients/animals with diseases selected from the group
consisting of degenerative diseases, cancer diseases,
autoimmune and/or inflammatory diseases, cardiovascular
diseases and neurological disorders.
5. The screening method according to claim 4, wherein
the test cells are selected from the group consisting
of Jurkat, HeLa, A20, KB, MCF7, Ramos, SK-MEL-1, SK-
MEL-28, PC-3, NCI-H460, NCI-H1792, Raji, SK-BR-3, HaCaT,
DM, HBL, SW480, HT-1080, HBL-100, Hs578T, MDA-MB-330,
C-33A, BT-474, MDA-MB-133-VI, MDA-MB-157, MOLT-4, K-
562, HCT-8, SW620, SW480, LoVo, SW403, SW1471, HL-60,
HUT 78, H9, U937, Hep G2, PLC/PRF/5, Hs 683, U-138MG,
A172 cells.
6. The screening method according to claim 4, wherein said
test cells are spheroids.
7. The screening method according to claims 1 to 6,
wherein said vectors are selected from the group
consisting of pEGFP-N1+MoLV-LTR, pBluescriptIIKS(+)+EF-

1 α -EGFP and any commercially available suitable vectors.

- 5 8. The screening method according to claim 7, wherein the primary screening step comprises measurement of the overall fluorescence activity of the test cells within a single well with a fluorescence detecting device.
- 10 9. The screening method according to claim 8, wherein the fluorescence detecting device is a fluorescence plate reader.
- 15 10. The screening method according to claim 9, wherein the primary screening step discriminates between two groups of different activities.
- 20 11. The screening method according to claim 10, wherein the primary screening step discriminates on one side between pro-apoptotic and/or necrotic activity and on the other side non- and/or anti-apoptotic and/or proliferative activity of test compounds.
- 25 12. The screening method according to claim 11, wherein the secondary screening step comprises measuring of single-cell fluorescence activity of each test cell within a population of test cells with a fluorescence detection device.
- 30 13. The screening method according to claim 12, wherein the fluorescence detecting device is selected from the group consisting of flow cytometry, microfluid (chip) devices, and single cell imaging scanning systems.
- 35 14. A high throughput screening method for assaying non-, pro- or anti-apoptotic or proliferative or necrotic activity of test compounds in cells using vectors coding for a marker protein and cells transfected with said vector comprising a single screening step as screening step for the discrimination between non-,

pro-, or anti-apoptotic, or proliferative or necrotic activity of compounds to be tested.

5 15. The screening method according to claim 14, wherein the primary screening step is carried out with a single cell imaging scanning system.

10 16. The screening method according to any of the preceding claims, wherein the compound to be tested is selected from the group consisting of synthetic or natural compounds, chemical or peptide structures or a combination thereof, proteins or recombinant proteins, pure compounds or a combination of pure compounds or extracts, such as plant extracts, extracts of marine
15 micro- and macro-organisms and extracts of microbial fermentations.

20 17. The screening method according to claim 16, wherein the compound to be tested is a therapeutic/diagnostic agent selected from the groups comprising:

- a) antimetabolites;
- b) alkylating agents;
- c) cell-cycle inhibitors;
- d) DNA breaker (topo-isomerase inhibitor, intercalator,
15 strand breaker);
- e) mixtures thereof;
- f) compounds interfering with the signal transduction pathway, such as caspase activity modifiers, agonists and antagonists of cell death receptors,
20 modifiers of nucleases, phosphatases and kinases.

5 18. The screening method according to any of the preceding claims, wherein the test cell system comprises cell lines or primary cells in form of single cells, single-cell populations of same origin, mixed-cell populations of different origin or spheroid cell forms.

19. The screening method according to claim 18, wherein the test cell system comprises eukaryotic cells selected from the group consisting of mammalian, fungal, insect, avian, worm, fish, crustacean, reptilian, amphibian and plant cells.
20. The screening method according to claim 19, wherein said eukaryotic cells are genetically non-altered cells, cells infected with virus, parasites, bacteria, fungi or prions, tumor cells or genetically manipulated or altered cells of human, animal or plant origin.
21. The screening method according to claim 20, wherein said eukaryotic cells are derived from human or animal tissues and/or organs selected from the group comprising liver, kidney, spleen, heart, lung, brain, blood, skin, muscles, bladder, myeloid and lymphoid system, reproductive system, bone marrow, gut, small intestine, mucosa, stomach, esophagus, duodenum, colon, pancreas, connective, embryonal and fetal tissue.
22. The screening method according to claim 21, wherein the test cell system is applied as healthy tissue- and/or organ- model or disease tissue model.
23. The screening method according to claims 1 to 18, wherein the test cell system comprises prokaryotic cells selected from the group consisting of bacterial and cyanobacterial cells.

24. The method according to any of the preceding claims, wherein the microtiter formate comprises a 96, 384, 1536 or any intermediate formate well plate or microchip technology.
- 5 25. Use of the screening method according to any of the preceding claims for drug screening.
- 10 26. The use of the screening method according to claim 25, wherein the drug screening is applied in the therapeutic and diagnostic fields selected from the group comprising cancer including angiogenesis, autoimmune and transplantation derived diseases, cardiovascular and degenerative diseases of various
15 origin, such as neurodegenerative diseases, inflammation and allergic diseases, diseases of the reproductive system, dermatological applications and related diseases.
- 20 27. Use of the screening method according to claims 1 to 24 for toxicological studies.
28. The use of the screening method according to claim 27, comprising an assaying of necrotic activity of
5 toxic compounds.
29. The use of the screening method according to claim 28, the toxicological studies being selected from the group comprising hepatotoxicological, kidney toxicity, skin toxicity, neurotoxicity, connective,
0 embryonal and fetal toxicity studies, toxicity of the spleen, heart, lung, blood, skin, muscles, bladder, myeloid and lymphoid system, reproductive system, visual system, bone marrow, gut, small

intestine, mucosa, stomach, esophagous, duodenum,
colon and pancreas.